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NUCLEOPHILIC ADDITION OF ALIPHATIC AMINO ACIDS TO N-2-METHYL-9-HYDROXY-ELLIPTICINIUM ACETATE UNDER PEROXIDASE-CATALYSED OXIDATIVE CONDITIONS : A REINVESTIGATION AND STRUCTURAL REVISION OF THE ADDUCTS

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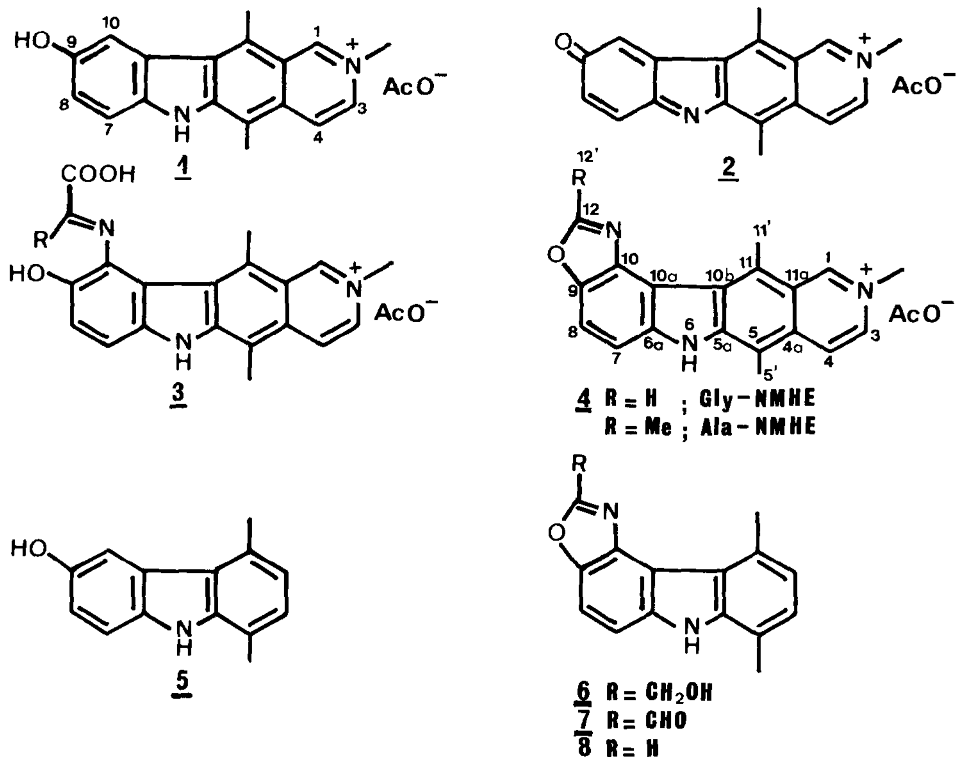
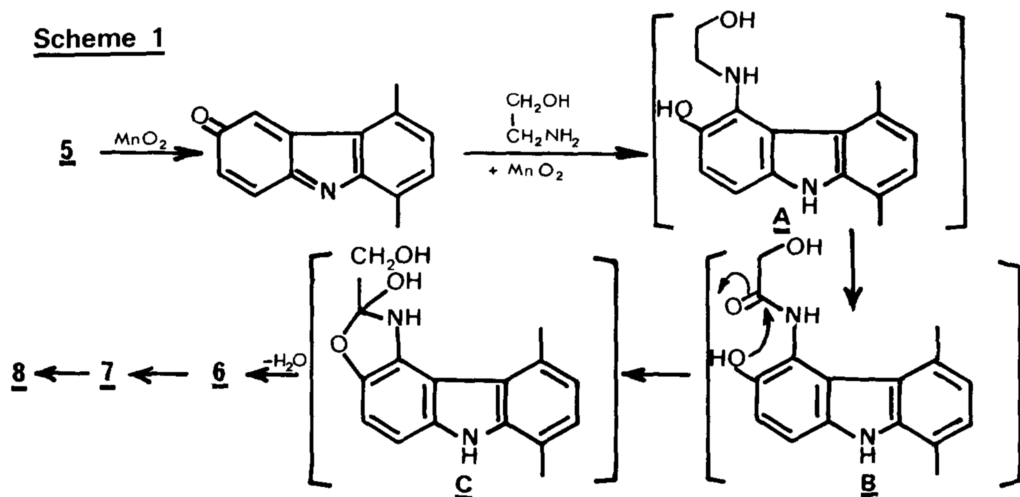
Summary : *A reinvestigation of the adducts resulting from a peroxidase-catalysed reaction of N-2-methyl-9-hydroxyellipticinium acetate 1 with some aliphatic amino acids led to their structural revision from 3 to 4.*

N-2-Methyl-9-hydroxyellipticinium (NMHE) acetate 1, a derivative of the indole alkaloid ellipticine, is known for its high cytotoxic activity[1] and is currently employed in the treatment of osteolytic metastases of breast cancer[2]. The quinone-imine system present in its oxidised form 2 is prone to regioselective Michael type addition reactions with various nucleophiles providing different 10-substituted derivatives[3,4]. The efficient synthesis of such adducts is of significance not only for understanding the in vivo mechanism of action of this drug, but also for an evaluation of the structure-activity relationships with a view to obtain more potent therapeutic agents. In this context, the synthesis of several aliphatic amino acid (glycine, alanine, valine, leucine) conjugates of NMHE acetate 1 under peroxidase-catalysed oxidative conditions (Horse Radish Peroxidase - H_2O_2) has recently been reported by Auclair et al.[5]. However, it seemed to us that the structures 3 assigned to these amino acid adducts AA-NMHE (Gly-NMHE, R = H ; Ala-NMHE, R = Me ; Val-NMHE, R = $CHMe_2$; Leu-NMHE, R = CH_2CHMe_2) rest on dubious spectral interpretation. Thus, the occurrence of an absorption band at $1670 cm^{-1}$ in their i.r. spectra was considered as evidence for the presence of a free carboxyl group, although these addition products were isolated as the acetate salts. Furthermore, the highest mass peaks observed in their desorption chemical ionisation (using NH_3) mass spectra were 46 mass units (m.u.) lower than the expected mass numbers of the cations corresponding to formulation 3 and this was explained as due to loss of carbon dioxide (-44 m.u.) from the carboxyl group along with the oxidation (-2 m.u. ; loss of 2H) of the hydroxyindole function to the corresponding quinone-imine. But, whereas decarboxylation ($-CO_2$) in the mass spectrometer is not an unknown phenomenon, the oxidation (-2H) of the hydroxyindole to the quinone-imine under chemical ionisation conditions is, to our knowledge, without precedent.

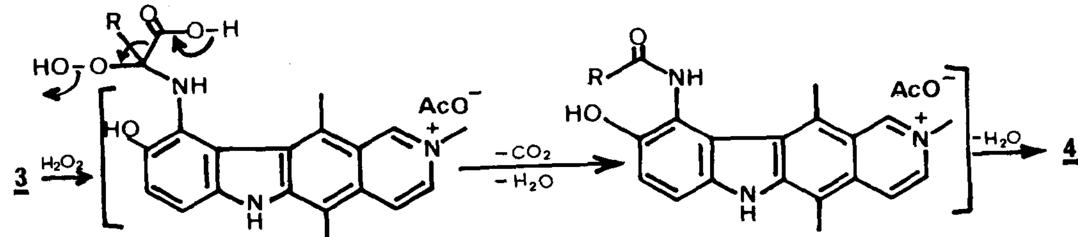
The need for reinvestigation and unambiguous structural determination of these amino acid adducts AA-NMHE therefore became mandatory. Herein we present arguments,



Scheme 1



Scheme 2



together with supporting experimental evidence, leading to structural revision 4 for such derivatives.

In order to address the problem, the Gly-NMHE and Ala-NMHE adducts were prepared as the acetate salts according to the published procedure[5] and their identity established from the ^1H n.m.r. and mass spectral data. The possible interference of the acetate ions in the carbonyl absorption region of the i.r. spectra was subsequently eliminated by their conversion[3] into the corresponding PF_6^- salts. Instead of a band at 1670 cm^{-1}, we now noted in the i.r. spectra of both the PF_6^- salts only a band of medium intensity at 1640 cm^{-1} which could be readily attributed to the -C=N- absorption. The absence of a carbonyl band rendered the previously assigned structures 3 for Gly-NMHE (R = H) and Ala-NMHE (R = Me) adducts unacceptable. It became clear that decarboxylation took place during the nucleophilic addition of the amino acids to NMHE acetate 1 under the oxidation condition employed. Also, the recognition[6] that these AA-NMHE conjugates resisted further oxidation excluded the oxidation-prone hydroxyindole structure as presented in 3.

In light of the above observations the previously reported[5] mass spectral data m/z 302 and m/z 316 (also confirmed by Fast Atom Bombardment mass spectrometry in the present study) corresponding to the cation of Gly-NMHE and Ala-NMHE adducts, respectively, were reevaluated and found to be compatible with structure 4 (R = H and Me) containing a fused oxazole ring system. The published ^1H n.m.r. data as well as our newly obtained ^{13}C n.m.r. data[7] are also fully consistent with the revised formulation 4. As a consequence of this structural revision, the one-proton singlet at δ 8.34 in the ^1H n.m.r. spectrum[5] of Gly-NMHE (4 ; R = H) should now be attributed to the oxazole ring proton.

Further support is lent to the fused oxazole ring assignment 4 through a finding which resulted from a separate but related investigation involving an analogous nucleophilic addition to the hydroxycarbazole derivative 5[8], albeit using a different oxidative system. Thus, the reaction of 5 with ethanolamine (5 molar equiv.) in dichloromethane solution in the presence of a five-fold excess of active manganese dioxide provided, after chromatographic separation and purification, three products 6, 7 and 8 in 19, 7 and 45 % yield, respectively. The structures of these compounds could be unambiguously assigned on the basis of their spectral analyses full details of which will be disclosed elsewhere. A plausible mechanism for the formation of 6, 7 and 8 through the intermediacy of A - C is depicted in Scheme 1. The known oxidative demethylation[9] of *N*,*N*-dimethylaniline to *N*-methylaniline via *N*-formyl-*N*-methylaniline by using manganese dioxide as an oxidant led us to consider the species B as an intermediate in this mechanistic rationale.

The aforementioned results suggested that a similar mechanism should also explain the formation of the AA-NMHE adducts 4 via oxidative decarboxylation of 3 followed by ring closure and dehydration (Scheme 2) under the peroxidase-catalysed reaction conditions.

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References

1 J.B. Le Pecq, C. Gosse, N. Dat-Xuong and C. Paoletti, C. R. Hebd. Seances Acad. Sci., 1975, 281, 1365 ; C. Paoletti, S. Cros, N. Dat-Xuong, P. Lecointe, and A. Moisand, Chem. Biol. Int., 1984, 25, 45.

2 P. Juret, A. Tanguy, J.Y. Le Talaer, J.S. Abatucci, N. Dat-Xuong, J.B. Le Pecq and C. Paoletti, Eur. J. Cancer, 1978, 14, 205 ; C. Paoletti, J.B. Le Pecq, N. Dat-Xuong, P. Juret, H. Garnier, H. Amiel and J. Rouesse, Recent Results Cancer Res., 1980, 74, 107 ; P. Juret, J.F. Heron, J.E. Couette, T. Delozier and J.Y. Le Talaer, Cancer Treat. Rep., 1982, 66, 1909 ; A. Clasysse, A. Brugarolas, P. Siegenthaler, R. Abele, F. Cavalli, R. De Jager, G. Renard, M. Roseneweig and H.H. Hansen, Eur. J. Cancer Clin. Oncol., 1984, 20, 243.

3 G. Meunier, B. Meunier, C. Auclair, J. Bernadou and C. Paoletti, Tetrahedron Lett., 1983, 24, 365.

4 V.K. Kansal, S. Funakoshi, P. Mangeney, P. Potier, B. Gillet, E. Guittet and J.-Y. Lallemand, Tetrahedron Lett., 1984, 25, 2351 ; G. Pratviel, J. Bernadou and B. Meunier, J. Chem. Soc., Chem. Commun., 1985, 60 ; V.K. Kansal, P. Potier, G. Gillet, E. Guittet, J.-Y. Lallemand, T. Huynh-Dinh and J. Igolen, Tetrahedron Lett., 1985, 26, 2891.

5 C. Auclair, E. Voisin, H. Banoun, C. Paoletti, J. Bernadou and B. Meunier, J. Med. Chem., 1984, 27, 1161.

6 B. Meunier, C. Auclair, J. Bernadou, G. Meunier, M. Maftouh, S. Cros, B. Monsarrat and C. Paoletti in "Structure-Activity Relationships of Antitumor Agents", Eds. D.N. Reinhoudt, T.A. Connors, H.M. Pinedo and K.W. Van De Poll, Martinus and Nijhoff Publishers, The Hague/Boston/London, 1983, pp. 149-181.

7 ^{13}C nmr data (CD_3OH + D_2O + CD_3COOD, 50.3 MHz) : 4 (R = H), δ 150.9 (C-1), 51.38 (N^2-Me), 135.6 (C-3), 116.39^a (C-4), 130.7 (C-4a), 115.03 (C-5), 15.59 (C-5'), 144.93 (C-5a), 138.96 (C-6a), 124.61 (C-7), 114.15^a (C-8), 150.28 (C-9), 148.88 (C-10), 117.36 (C-10a), 136.8^b (C-10b), 125.09 (C-11), 20.89 (C-11'), 138.7^b (C-11a), 157.58 (C-12) ; 4 (R = Me), δ 150.98 (C-1), 51.32 (N^2-Me), 136.46 (C-3), 116.0^c (C-4), 131.51 (C-4a), 115.01 (C-5), 15.68 (C-5'), 145.7 (C-5a), 141.08 (C-6a), 125.57 (C-7),113.46^c (C-8), 151.64 (C-9), 149.9 (C-10), 117.32 (C-10a), 138.11^d (C-10b), 125.57 (C-11), 20.96 (C-11'), 139.43^d (C-11a), 169.46 (C-12), 18.98 (C-12') ; *a, b, c, d :* these assignments may be interchanged.

8 A. Ahond, C. Poupat and P. Potier, Tetrahedron, 1978, 34, 2385.

9 H.B. Henbest and A. Thomas, J. Chem. Soc., 1957, 3032.

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